OMB Number: 4040-0001 Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE	3. DATE RECEIVED BY STATE State Application Identifier							
SF 424 (R&R)	State Application Identifier							
1. * TYPE OF SUBMISSION	4. a. Federal Identifier N00014							
Pre-application Application Changed/Corrected Application	b. Agency Routing Identifier 342 [Chrisey, Linda A]							
2. DATE SUBMITTED Applicant Identifier								
F APPLICANT INFORMATION								
5. APPLICANT INFORMATION	* Organizational DUNS: 1539267120000							
* Legal Name: University of Massachusetts Amherst Department: Grant and Contract Admin Division: R.	, , , , ,							
Department: Grant and Contract Admin. Division: R. * Street1: 70 Butterfield Terrace	esearch and Engagement							
Street2: Research Administration Bldq								
* City: Amherst County / Pa	ırish:							
* State: MA: Massachusetts	Province:							
* Country: USA: UNITED STATES	* ZIP / Postal Code: 01003-9242							
Person to be contacted on matters involving this application								
Prefix: Ms. * First Name: Carol	Middle Name: P.							
* Last Name: Sprague	Suffix:							
* Phone Number: 413-545-0698 Fax Number: 413	3-545-1202							
Email: ogca@research.umass.edu								
6. * EMPLOYER IDENTIFICATION (EIN) or (TIN): 043167352								
7.* TYPE OF APPLICANT: H: Public/State	Controlled Institution of Higher Education							
Other (Specify):								
Small Business Organization Type Women Owned So	cially and Economically Disadvantaged							
	c appropriate box(es).							
	Award B. Decrease Award C. Increase Duration D. Decrease Duration							
Renewal Continuation Revision E. Other (s								
* Is this application being submitted to other agencies? Yes No	What other Agencies?							
	ALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: 12.300							
Office of Naval Research	Basic and Applied Scientific Research							
11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:								
Factors Limiting Power Output of Benthic Microbial Fu	el Cells: Mechanistic and Ecological Studies							
12. PROPOSED PROJECT: * 13. CONGRESSIONAL DISTR	ICT OF APPLICANT							
* Start Date								
03/01/2013 02/29/2016 MA-001								
14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INF Prefix: Dr. * First Name: Derek	-ORMATION Middle Name:							
* Last Name: Lovley	Suffix:							
Position/Title: Professor	Guinx.							
* Organization Name: University of Massachusetts Amherst								
	esearch and Engagement							
* Street1: 639 North Pleasant St								
Street2: 203N Morrill IVN								
* City: Amherst County / Pa	arish:							
* State: MA: Massachusetts	Province:							
* Country: USA: UNITED STATES	* ZIP / Postal Code: 01003-9298							
* Phone Number: 413-545-9651 Fax Number: 413	3-577-4660							
* Email: dlovley@microbio.umass.edu	<u> </u>							

15. ESTIMATED PROJECT FUNDING	9	16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?								
a. Total Federal Funds Requested b. Total Non-Federal Funds c. Total Federal & Non-Federal Funds d. Estimated Program Income	on-Federal Funds olderal & Non-Federal Funds olderal & Non									
17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001) * I agree * The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions. 18. SFLLL or other Explanatory Documentation										
18. SFLLL or other Explanatory Doc	umentation		Add Attachmer	nt D	elete Attachment	View Attachment				
19. Authorized Representative										
Prefix: Ms. * First N	Name: _{Carol}			Middle I	Name: P.					
* Last Name: Sprague				Suffix:						
* Position/Title: Director, Grant a	and Contract Administrat	ion								
* Organization: University of Mas		<u>'</u>								
Department: Grant and Contract	ct Admin Division:	Research	and Engagem	ent						
* Street1: 70 Butterfield Te	errace									
Street2: Research Administ	tration Bldg									
* City: Amherst	County / Par	rish:								
* State:	IA: Massachusetts		Provinc	e:						
* Country:	JSA: UNITED STATES		* ZIP / P	ostal Cod	e: 01003-9242					
* Phone Number: 413-545-0698	Fax Number: 4	113-545-12	02							
* Email: OGCA@research.umass.ed	du									
* Signature of Auth	orized Representative				* Date Signed	i				
	ol sprague				12/18/201	2				
20. Pre-application			Add Attachme	ent	Delete Attachment	View Attachment				

OMB Number: 4040-0001 Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

			TLOE/ III OII	¬	20 000021 0201	.o.,	JOL		•			
		1539267120000										
* Budget	Type: 🔀 Project	Subaward	d/Consortium									
Enter nar	ne of Organization	n: University of	Massachusetts .	Amh								
Delete l	Entry * Start	Date: 03/01/2013	* End Date: 09/30)/2013 B ı	udget Period 1							
Senior/K	ey Person						Cal.	Acad.	Sum.	* Requested	* Fringe	
Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)			Months		Benefits (\$)	* Funds Requested (\$)
Dr.	Derek		Lovley		PD/PI	(b) (4)			0.35	(b) (4)	(b) (4)	(b) (4)
Dr.	Kelly		Nevin		CoPI	(b) (4)		2.30		(b) (4)	(b) (4)	(b) (4)
Addition	al Senior Key Per	sons:			Add Attachment	Delete Attack	hment	View	Attachme	ent		
B. Other	Personnel											
	nber of sonnel		* [Project Role			Cal. Months	Acad. Months	Sum. s Month	•	* Fringe Benefits (\$)	* Funds Requested (\$)
1	Post [Doctoral Associates					3.50			(b) (4)	(b) (4)	(b) (4)
	Gradu	ate Students										
		graduate Students										
	Secre	tarial/Clerical						<u> </u>		_		
										<u> </u>	_	
	_]][
	=							1				
1								II		II .		
]][][<u> </u>		
1	Total	Number Other Persor	nnel							Tota	Other Personne	el (b) (4)
1	Total	Number Other Persor	nnel				Total	Salany	Wages		I Other Personne	(2) (.)

1. 2. 3. 4. 5. 6. 7. 8. 9.

RESEARCH & RELATED BUDGET - SECTION	N C, D, & E, BUDGET PERIOD 1
* ORGANIZATIONAL DUNS: 1539267120000	
* Budget Type: Project Subaward/Consortium	
Enter name of Organization: University of Massachusetts Amh	
Delete Entry * Start Date: 03/01/2013 * End Date: 09/30/2013 Budget	et Period 1
C. Equipment Description	
List items and dollar amount for each item exceeding \$5,000	
Equipment item	* Funds Requested (\$)
1.	
2.	
3.	
4.	
5.	
6	
8.	
9.	
10.	
11. Total funds requested for all equipment listed in the attached file	
Total Equipm	oment
Additional Equipment:	Add Attachment Delete Attachment View Attachment
Additional Equipment.	Add Attachment Delete Attachment View Attachment
D. Travel	Funds Requested (\$)
Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	2,000.00
	rel Cost 2,000.00
	2,000.00
E. Participant/Trainee Support Costs	Funds Requested (\$)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Number of Participants/Trainees Total Participant/Trainee Support	t Costs

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RE	LATED BUDGE	1 - SECTION F	r-k, bube	SET PERIOD T	Next Period
* ORGANIZATIONAL DUNS: 1539267120000					
* Budget Type: Project Subaward/0	Consortium				
Enter name of Organization: University of Ma	assachusetts Am	h			
	nd Date: 09/30/2		od 1		
5575172013	05/30/2	013			
F. Other Direct Costs			Funds Req	uested (\$)	
Materials and Supplies			8,079.00		
2. Publication Costs			0,075.00		
3. Consultant Services					
4. ADP/Computer Services					
5. Subawards/Consortium/Contractual Costs					
6. Equipment or Facility Rental/User Fees					
7. Alterations and Renovations					
8.					
9.					
10.					
	Total Othe	er Direct Costs	8 079 00		
	Total Oth	or Direct Goots	0,079.00		
G. Direct Costs			Funds Req		
	Total Direct C	osts (A thru F)	69,931.0	0	
H. Indirect Costs	Indirect Cost	Indirect Cost			
Indirect Cost Type	Rate (%)	Base (\$)	* Funds Re	quested (\$)	
1. Modified Total Direct Costs	59.00	9,931.00	41,259.0	0	
2.					
3.					
4.					
	Total	Indirect Costs	41,259.0	0	
Cognizant Federal Agency DHHS, Micheal Sta	ungo 212-264-1	823			
(Agency Name, POC Name, and POC Phone Number)		023			
I. Total Direct and Indirect Costs			Funds Req	uested (\$)	
Total Direct and Indirect In	stitutional Costs (G + H)	111,190.	00	
J. Fee			Funds Rec	uested (\$)	
J. Fee			Funds Req	uested (\$)	
J. Fee			Funds Req	uested (\$)	
J. Fee K. * Budget Justification BudgetJustification	SedimentMFC.p	df Add Atta		Delete Attachment	View Attachment

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001 Expiration Date: 06/30/2011

Previous	Period		RESEARCH	& RELAT	ED BUDGET - SECT	ION A & B, BU	DGET I	PERIO	2		ΣΑΡΙ	Tallott Bato. 00/00/2011
* ORGAN	NIZATIONAL DUNS	5: 1539267120000										
* Budget	Type: Project	Subawar	d/Consortium									
Enter na	me of Organization	n: University of	Massachusetts A	Amh								
Delete	* Start	Date: 10/01/2013	* End Date: 09/30	/2014 Bu	udget Period 2							
. Senior/	Key Person						Cal	A I	C	* D	* Faire are	
Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months	Acad. Months		* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$
Dr.	Derek		Lovley		PD/PI	(b) (4)			0.60	(b) (4)	(b) (4)	(b) (4)
Dr.	Kelly		Nevin		CoPI	(b) (4)		4.00		(b) (4)	(b) (4)	(b) (4)
Total Fu	nds requested for	all Senior Key Pers	ons in the attached	file								
										Total Se	nior/Key Person	(b) (4)
Addition	nal Senior Key Per	sons:			Add Attachment	Delete Attac	hment	View	Attachm	ent		
	•											
B. Other	Personnel											
* Nu	mber of						Cal.	Acad.	Sum.	* Requested	* Fringe	
Per	sonnel		* F	Project Role			Months	Months	Month	s Salary (\$)	Benefits (\$)	* Funds Requested (\$
1	Post D	Ooctoral Associates					6.00			(b) (4)	(b) (4)	(b) (4)
	Gradu	ate Students										
	Under	graduate Students										
	Secret	tarial/Clerical										
1	Total I	Number Other Perso	nnel							Tota	l Other Personne	el (b) (4)
							Total S	Salarv.	Wages	and Fringe	Benefits (A+E	3) (b) (4)

2.

5.6.7.8.9.

	RESEARCH & RELATED BUDGET - SECT	ION C, D	, & E, BUD	GET PERIOD 2	
* ORG	GANIZATIONAL DUNS: 1539267120000				
* Bud	get Type: Project Subaward/Consortium				
Enter	name of Organization: University of Massachusetts Amh				
Delet	te Entry * Start Date: 10/01/2013 * End Date: 09/30/2014 Bu	dget Perio	d 2		
C. Ed	quipment Description				
List i	items and dollar amount for each item exceeding \$5,000				
	Equipment item	,	* Funds Requ	ested (\$)	
1. [
2. [
3. [
4.					
5.					
6.					
7.					
8. [9. [
9. 10. [
L	Total funds requested for all equipment listed in the attached file				
• • • •		quipment			
A .d .				D 1 4 4 4 1 4 1	\(\text{\tin}\text{\text{\text{\text{\text{\text{\text{\text{\text{\text{\tint{\text{\tin}\text{\texi\tint{\text{\text{\text{\text{\text{\tin}}\tint{\text{\text{\text{\text{\text{\text{\text{\text{\text{\tin}}\tint{\text{\text{\tinit}\\ \tint{\text{\text{\text{\text{\text{\text{\text{\text{\tin}\tint{\text{\text{\texi}\tint{\text{\texi}\tint{\text{\ti}\titt{\text{\text{\text{\text{\texi}\tint{\text{\texi}\tint{\text{\texi}\tittit{\texititt{\text{\tinit}\titt{\tinit}\titt{\ti
Add	litional Equipment:	Add At	ttachment	Delete Attachment	View Attachment
D. Tr	ravol		Funds Requ	ostod (\$)	
	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)				
	Foreign Travel Costs		4,048.00		
		ravel Cost	4,048.00		
			4,048.00		
E. Pa	articipant/Trainee Support Costs		Funds Requ	ested (\$)	
1.	Tuition/Fees/Health Insurance				
2.	Stipends				
3.	Travel				
	Subsistence				
5.	Other				
	Number of Participants/Trainees Total Participant/Trainee Supp	port Costs			

RESEARCH & RELATED Budget {C-E} (Funds Requested)

* ORGANIZATIONAL DUNS: 1539267120000 * Budget Type: Project Subaward/0				
* Budget Type: Project Subaward/0				
- I Tojoot Oabawarara	Consortium			
Enter name of Organization: University of Ma	assachusetts	Amh		
	nd Date: 09/3		iod 2	
Delete Littly	09/3	0/2014] = 3.3		
F. Other Direct Costs			Funds Requested (\$)	
Materials and Supplies				
Publication Costs			15,127.00	
Consultant Services			2,020.00	
ADP/Computer Services				
Subawards/Consortium/Contractual Costs				
Equipment or Facility Rental/User Fees				
7. Alterations and Renovations				
8.				
9.				
10.				
10.				
	Total C	Other Direct Cost	S 17,147.00	
G. Direct Costs			Funds Requested (\$)	
O. D. Book Cooks	Total Direc	t Costs (A thru F		
	TOtal Direc	i Costs (A tillu i	127,524.00	
H. Indirect Costs	Indirect Cos	t Indirect Cost		
Indirect Cost Type	Rate (%)	Base (\$)	* Funds Requested (\$)	
Indirect Cost Type 1. Modified Total Direct Cost	Rate (%)		* Funds Requested (\$) 75,239.00	
	. ,	Base (\$)	1	
1. Modified Total Direct Cost	. ,	Base (\$)	1	
1. Modified Total Direct Cost 2.	. ,	Base (\$)	1	
1. Modified Total Direct Cost 2. 3.	59.00	Base (\$)	75,239.00	
1. Modified Total Direct Cost 2. 3. 4.	59.00	Base (\$)	75,239.00	
1. Modified Total Direct Cost 2. 3.	59.00	Base (\$)	75,239.00	
1. Modified Total Direct Cost 2. 3. 4. DHHS, Micheal Sta	59.00	Base (\$)	75,239.00	
1. Modified Total Direct Cost 2. 3. 4. DHHS, Micheal Sta	59.00	Base (\$)	75,239.00 	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal State (Agency Name, POC Name, and POC Phone Number)	59.00 To	Base (\$) 127,524.00 otal Indirect Costs 4-1823	75,239.00 S 75,239.00 Funds Requested (\$)	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs	59.00 To	Base (\$) 127,524.00 otal Indirect Costs 4-1823	75,239.00 	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs	59.00 To	Base (\$) 127,524.00 otal Indirect Costs 4-1823	75,239.00 S 75,239.00 Funds Requested (\$)	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs	59.00 To	Base (\$) 127,524.00 otal Indirect Costs 4-1823	75,239.00 S 75,239.00 Funds Requested (\$)	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs Total Direct and Indirect Indianal State (Agency Name)	59.00 To	Base (\$) 127,524.00 otal Indirect Costs 4-1823	75,239.00 75,239.00 75,239.00 Funds Requested (\$) 202,763.00	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs Total Direct and Indirect Indianal State (Agency Name)	59.00 To	Base (\$) 127,524.00 otal Indirect Costs 4-1823	75,239.00 75,239.00 75,239.00 Funds Requested (\$) 202,763.00	
1. Modified Total Direct Cost 2. 3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs Total Direct and Indirect Indianal State (Agency Name)	To anco, 212-26	Base (\$) 127,524.00 otal Indirect Cost: 4-1823	75,239.00 75,239.00 75,239.00 Funds Requested (\$) 202,763.00	View Attachment

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001 Expiration Date: 06/30/2011

Previous	Period		RESEARCH	& RELAT	ED BUDGET - SECT	ION A & B, BU	DGET I	PERIO	3		·	
* ORGAN	NIZATIONAL DUNS	1539267120000)									
_	Type: X Project	<u> </u>	d/Consortium									
Enter na	me of Organization	n: University of	Massachusetts A	Amh								
Delete	Entry * Start	Date: 10/30/2014	* End Date: 09/30)/2015 B	udget Period 3							
. Senior/	Key Person											
Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months		Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$
Dr.	Derek		Lovley		PD/PI	(b) (4)			0.60	(b) (4) ((b) (4)
Dr.	Kelly		Nevin		CoPI	(b) (4)		4.00		(b) (4)	(b) (4)	(b) (4)
Total Fu	nds requested for a	all Senior Key Pers	sons in the attached	file								
										Total Se	enior/Key Person	(b) (4)
Addition	nal Senior Key Per	sons:			Add Attachment	Delete Attac	hment	View	Attachm	ent		
B. Other	Personnel											
	mber of						Cal.	Acad.				
Per	sonnel		* F	Project Role	•		Months	Months	Month	s Salary (\$)	Benefits (\$)	* Funds Requested (\$
1	Post D	octoral Associates					6.00			(b) (4)	(b) (4)	(b) (4)
	Gradu	ate Students										
	Under	graduate Students										
	Secret	arial/Clerical										
1	Total N	Number Other Perso	onnel							Tota	al Other Personn	el (b) (4)
							Total 9	Salary.	Wages	and Fringe	Benefits (A+F	3) (b) (d)

2.

5.

7. 8. 9.

	RESEARCH & RELATED BUDGET - SECT	ION C, D	, & E, BUD	GET PERIOD 3	
* ORG	GANIZATIONAL DUNS: 1539267120000				
* Bud	get Type: Project Subaward/Consortium				
Enter	name of Organization: University of Massachusetts Amh				
Delet	te Entry * Start Date: 10/30/2014 * End Date: 09/30/2015 Bu	dget Perio	d 3		
C. Ed	quipment Description				
List i	tems and dollar amount for each item exceeding \$5,000				
	Equipment item	,	* Funds Requ	uested (\$)	
1. [
2. [
3. [
4.					
5.					
6.					
7.					
8. [9. [
9. 10. [
L	Total funds requested for all equipment listed in the attached file				
• • • •		quipment			
A .1.					
Add	litional Equipment:	Add At	ttachment	Delete Attachment	View Attachment
D. Tr	avol		Funds Requ	ostad (\$)	
	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)				
	Foreign Travel Costs		4,129.00		
		ravel Cost	4,129.00		
			4,129.00		
E. Pa	articipant/Trainee Support Costs		Funds Requ	ested (\$)	
1.	Tuition/Fees/Health Insurance				
2.	Stipends				
3.	Travel				
	Subsistence				
5.	Other				
	Number of Participants/Trainees Total Participant/Trainee Supp	oort Costs			

RESEARCH & RELATED Budget {C-E} (Funds Requested)

INESERTION & INE	LATED BUD	OGET - SECTION	F-K, BUDGET PERIOD 3	Next Period
* ORGANIZATIONAL DUNS: 1539267120000			-	
* Budget Type: Project Subaward/0	Consortium			
Enter name of Organization: University of Ma		s Amh		
<u> </u>	ind Date: 09/3		riod 3	
F. Other Direct Costs			Funds Requested (\$)	
1. Materials and Supplies			15,430.00	
2. Publication Costs			2,060.00	
3. Consultant Services				
4. ADP/Computer Services				
5. Subawards/Consortium/Contractual Costs				
6. Equipment or Facility Rental/User Fees				
7. Alterations and Renovations				
8.				
9.				
10.				
	Total (Other Direct Cost	S 17,490.00	
G. Direct Costs			Funds Requested (\$)	
G. Direct Costs	Total Dira	ct Costs (A thru F		
	TOTAL DIFE	ci Costs (A tillu F	[131,118.00]	
H. Indirect Costs	Indirect Cos		* Funds Requested (\$)	
Indirect Cost Type	Rate (%)	Base (\$)	runas Requestea (\$)	
1. Modified Total Direct Cost	11		1	
	59.00	131,118.00	77,360.00	
2.	59.00] [131,118.00	77,360.00	
3.	59.00	131,118.00	77,360.00	
3	To	otal Indirect Cost		
3. 4. Cognizant Federal Agency DHHS, Micheal Sta	To	otal Indirect Cost		
3	To	otal Indirect Cost		
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number)	To	otal Indirect Cost	\$ 77,360.00	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs	To	otal Indirect Cost	\$ 77,360.00 Funds Requested (\$)	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number)	To	otal Indirect Cost	\$ 77,360.00	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs	To	otal Indirect Cost	\$ 77,360.00 Funds Requested (\$)	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs	To	otal Indirect Cost	\$ 77,360.00 Funds Requested (\$)	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs Total Direct and Indirect In	To	otal Indirect Cost	Funds Requested (\$) 208,478.00	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs Total Direct and Indirect In	To	otal Indirect Cost	Funds Requested (\$) 208,478.00	
3. 4. Cognizant Federal Agency DHHS, Micheal Sta (Agency Name, POC Name, and POC Phone Number) I. Total Direct and Indirect Costs Total Direct and Indirect In	To anco, 212-26	otal Indirect Cost	Funds Requested (\$) 208,478.00	View Attachment

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001 Expiration Date: 06/30/2011

Previous	Period		RESEARCH 8	& RELAT	ED BUDGET - SECT	ION A & B, BU	DGET I	PERIO) 4		r	
* ORGAN	NIZATIONAL DUNS	1539267120000)									
_	t Type: X Project	<u> </u>	d/Consortium									
Enter na	me of Organization	n: University of	Massachusetts A	mh								
Delete	Entry * Start	Date: 10/01/2015	* End Date: 02/29	/2016 B ı	udget Period 4							
. Senior/I	Key Person											
Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary (\$)	Cal. Months		Sum. Months	* Requested Salary (\$)	* Fringe Benefits (\$)	* Funds Requested (\$
Dr.	Derek		Lovley		PD/PI	(b) (4)			0.25	(b) (4)	(b) (4)	(b) (4)
Dr.	Kelly		Nevin		CoPI	(b) (4)		1.70		(b) (4)	(b) (4)	(b) (4)
Total Fu	nds requested for a	all Senior Key Pers	sons in the attached t	file								
										Total Se	nior/Key Person	(b) (4)
Addition	nal Senior Key Per	sons:			Add Attachment	Delete Attac	hment	View	Attachme	ent		
B. Other	Personnel											
	mber of						Cal.	Acad.		* Requested	* Fringe	
Per	rsonnel		* P	roject Role			Months	Months	Month	s Salary (\$)	Benefits (\$)	* Funds Requested (\$
1	Post D	Ooctoral Associates					2.50			(b) (4)	(b) (4)	(b) (4)
	Gradu	ate Students										
	Under	graduate Students										
	Secret	tarial/Clerical										
									<u> </u>			
1	Total N	Number Other Perso	onnel							Tota	l Other Personne	el (b) (4)
							Total 9	Salary.	Wages	and Fringe	Benefits (A+F	3) (b) (4)

2.

5.

7. 8. 9.

RESEARCH & RELATED BUDGET - SECTION	N C, D, 8	& E, BUD	GET PERIOD 4	
* ORGANIZATIONAL DUNS: 1539267120000				
* Budget Type: Project Subaward/Consortium				
Enter name of Organization: University of Massachusetts Amh				
Delete Entry * Start Date: 10/01/2015 * End Date: 02/29/2016 Budge	et Period	4		
C. Equipment Description				
List items and dollar amount for each item exceeding \$5,000				
Equipment item	* F	Funds Requ	ested (\$)	
1.				
2.				
3.				
4.				
5				
7.				
8.				
9.				
10.				
11. Total funds requested for all equipment listed in the attached file				
Total Equip	ment			
Additional Equipment:	Add Attac	chment	Delete Attachment	View Attachment
D. Travel	F	unds Reque	ested (\$)	
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1	L,088.00		
2. Foreign Travel Costs				
Total Trav	el Cost 1	L,088.00		
E. Participant/Trainee Support Costs	F	unds Reque	ested (\$)	
1. Tuition/Fees/Health Insurance				
2. Stipends				
3. Travel	L			
4. Subsistence	_,			
5. Other				
Number of Participants/Trainees Total Participant/Trainee Suppor	Costs			

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RE	LATED BUD	GET - SECTION I	F-K, BUDGET PERIOD 4	Next Period
* ORGANIZATIONAL DUNS: 1539267120000				
* Budget Type: Project Subaward/0	Consortium			
Enter name of Organization: University of Ma		7 mh		
	nd Date: 02/2		od 4	
Delete Entry Start Date: 10/01/2015 * El	02/2	9/2016 Budget Fell	ou 4	
F. Other Direct Costs			Funds Requested (\$)	
1. Materials and Supplies			6,988.00	
2. Publication Costs			1,088.00	
3. Consultant Services				
4. ADP/Computer Services				
5. Subawards/Consortium/Contractual Costs				
6. Equipment or Facility Rental/User Fees				
7. Alterations and Renovations				
8.				
9.				
10.				
	Total O	ther Direct Costs		
	Total O	ther Direct Costs	8,076.00	
G. Direct Costs			Funds Requested (\$)	
	Total Direc	t Costs (A thru F	56,561.00	
H. Indirect Costs	Indirect Cost	t Indirect Cost		
Indirect Cost Type	Rate (%)	Base (\$)	* Funds Requested (\$)	
1. Modified Total Direct Cost	59.00	56,561.00	33,371.00	
2.				
3.				
4.				
	То	tal Indirect Costs	33,371.00	
Cognizant Federal Agency DHHS, Micheal Sta	ngo 212-26	4_1823		
(Agency Name, POC Name, and POC Phone Number)	1100, 212 20	1 1023		
I. Total Direct and Indirect Costs			Funds Requested (\$)	
Total Direct and Indirect In	stitutional Cos	ts (G + H)	89,932.00	
J. Fee			Funds Requested (\$)	
K. * Budget Justification BudgetJustification	SedimentMF	and Add Att	achment Delete Attachment	View Attachment

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals	s (\$)
Section A, Senior/Key Person		(b) (4)
Section B, Other Personnel		(b) (4)
Total Number Other Personnel	4	
Total Salary, Wages and Fringe Benefits (A+B)		(b) (4)
Section C, Equipment		
Section D, Travel		11,265.00
1. Domestic	11,265.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		50,792.00
1. Materials and Supplies	45,624.00	
2. Publication Costs	5,168.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		385,134.00
Section H, Indirect Costs		227,229.00
Section I, Total Direct and Indirect Costs (G + H)		612,363.00
Section J, Fee		

BUDGET JUSTIFICATION

Overall Budget:

Period 1 (3/1/13 - 9/30/13): \$111,190 Period 2 (10/1/13 - 9/30/14): \$202,763 Period 3 (10/1/14 - 9/30/15): \$208,478 Period 4 (10/1/15 - 2/29/16): \$89,932 Total Funding (3/1/13 - 2/29/16): \$612,363

Personnel:

Funds are requested for 0.6 months summer salary each calendar year for the principal investigator to coordinate experimental approaches and to prepare reports and peer-reviewed articles for the project.

Period 1 @ 0.35 months

Period 2 @ 0.6 months

Period 3 @ 0.6 months

Period 4 @ 0.25 months

Rate is based on current salary plus 3% COLA per budget period. (b) (4) total funding)

Funds are requested for 4 months academic salary each calendar year for the co-principal investigator to develop novel aspects of the experimental approaches and carry out those experiments requiring substantial prior experience with microbial fuel cells as well as to supervise the postdoctoral research associate on a daily basis.

Period 1 @ 2.3 months

Period 2 @ 4 months

Period 3 @ 4 months

Period 4 @ 1.7 months

Rate is based on current salary plus 3% COLA per budget period. (b) (4) total funding)

Funds are further requested for one part-time postdoctoral research associate (6 calendar months each calendar year) to conduct genetics studies and other aspects of the research.

Period 1 @ 3.5 months

Period 2 @ 6 months

Period 3 @ 6 months

Period 4 @ 2.5 months

Rate is based on current NIH standards plus 3% COLA per budget period. (b) (4) total funding)

Fringe Rates:

Faculty PI (b) (4) total funding):

Workers Compensation 0.53%

Unemployment, Universal Health, MTX (Medicare tax) 1.29%

Faculty Co-PI (b) (4) total funding):

Fringe 25.98%

Workers Compensation 0.53%

Unemployment, Universal Health, MTX (Medicare tax) 1.29%

Health and Welfare \$14/week

Postdoctoral Fellow (b) (4) total funding):

Fringe 10.91%

Workers Compensation 0.53%

Unemployment, Universal Health, MTX (Medicare tax) 1.29%

Health and Welfare \$14/week

Rates are based on current negotiated and approved rates. (Attached)

Travel:

Funds are requested for travel to National Microbiology meetings to present data (\$2000/person/trip), and Washington DC for ONR meetings (\$1000/person/trip). Rates are based on previous experience with purchases for similar travel with 2% inflation rate.

Publications:

Funds are requested for publication costs in peer-reviewed journals each calendar year. (\$1000-2000/article depending on journal and size)

Rates are based on previous experience with purchases for similar publications with 2% inflation rate.

Materials and Supplies:

Funds for materials and supplies requested at an approximate rate of about \$18,000 per full time effort researcher (Co-PI and Postdoc) for each calendar year.

Rate is based on previous experience with purchases for similar research projects with 2% inflation rate.

Materials and Supplies details:

Supply Items include: Custom glassware; electrodes; anode and cathode graphite materials; selective membranes; wires, connectors and resistors; gasket materials; gassing station components: swage fittings, flow meters, pressure gauges; reagents for analytical and electrochemical analysis; gases for anaerobic culturing and fuel cells.

Transmission electron microscopy supplies including: labeled antibodies, support film/grids and electron microscopy use, probes for thermopower and high-frequency measurements; tips for electrostatic force microscopy; liquid helium and liquid nitrogen; specific fluorophores; miscellaneous reagents for molecular, analytical, electrochemical analyses.

Molecular Biology reagents and supplies: acidic phenol, isopropanol, ethanol, isoamyl alcohol/chloroform, TE saturated phenol, linear acrylamide; Superase-In, Proteinase K, lysozyme, yeast tRNA, glycogen, Rneasy mini kits; RNA isolation aid kit; DNA-free kit; reverse

transcriptase, restriction enzymes, primers, taq DNA polymerase, dNTPs; PCR primers; TOPO vector cloning kits; microarray supplies including RNA amplification kit and slide chips; DNA sequencing supplies including Big Dye terminator kit, POP7 polymer General laboratory reagents, supplies, and small equipment: gases for anaerobic glove bags, anaerobic culturing stations, and bench-top manipulations; columns and reagents for HPLC and ion and gas chromatographs; reagents for protein assays, disposable syringes, needles, pipette tips, filters, tubes, gloves, culturing tubes, butyl rubber stoppers, media ingredients; cell counting supplies and microscope supplies.

Indirect costs:

59.0% of total direct costs for 3/1/13-2/29/16 Rates are based on current negotiated and approved rates. (Attached)

Further details will be supplied if requested

COLLEGES AND UNIVERSITIES RATE AGREEMENT

EIN #: 043167352 DATE: July 8, 2009

INSTITUTION: FILING REF.: The preced:

University of Massachusetts at Amherst Agreement was dated

340 Whitmore Administration Bldg. June 26, 2008

181 Presidents Drive

Amherst MA 01003-9313

The rates approved in this agreement are for use on grants, contracts and other agreements with the Federal Government, subject to the conditions in Section III.

SECTION	I : FACILITI	ES AND ADM	INISTRATI	E COST RATES*	
RATE TY	PES: FIXED	FINAL	PROV. (I	PROVISIONAL)	PRED. (PREDETERMINED)
	EFFECTIVE	~			
TYPE	FROM	TO	RATE(%)	LOCATIONS	APPLICABLE TO
			(b) (4)		

TIPE	FROM	10	RAIE (*)	LOCATIONS	APPLICABLE TO
PRED.	07/01/10		(b) (4)	On-Campus On-Campus	Research Research
PRED. PRED.	07/01/11	06/30/12		On-Campus	Research
PRED.	07/01/12			On-Campus Off-Campus	Research Research
PRED.		06/30/13		On-Campus	Instruction
PRED.		06/30/13		Off-Campus	Instruction
PRED.		06/30/13		On-Campus	Other Sponsored Act.
PRED.		06/30/13		Off-Campus	Other Sponsored Act.
PROV.	07/01/13	UNTIL AMENDED	Ome same	rates and condi	tions as those cited

for fiscal year ending June 30, 2013.

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i '.

Modified total direct costs, consisting of all salaries and wages, fringe benefits, materials, supplies, services, travel and subgrants and subcontracts up to the first \$25,000 of each subgrant or subcontract (regardless of the period covered by the subgrant or subcontract). Modified total direct costs shall exclude equipment, capital expenditures, charges for patient care, student tuition remission, rental costs of off-site facilities, scholarships, and fellowships as well as the portion of each subgrant and subcontract in excess of \$25,000.

7:29AM

INSTITUTION:

University of Massachusetts at Amherst

AGREEMENT DATE: July 8, 2009

SECTION II: SPECIAL REMARKS

TREATMENT OF PAID ABSENCES:

Vacation, holiday, sick leave pay and other paid absences are included in salaries and wages and are claimed on grants, contracts and other agreements as part of the normal cost for salaries and wages. Separate claims for the costs of these paid absences are not made.

- 1. The rates in this Agreement have been negotiated to reflect the administrative cap provisions of the revisions to OMB Circular A-21 published by the Office of Management and Budget on May 8, 1996. No rate affecting the institution's fiscal periods beginning on or after October 1, 1991 contains total administrative cost components in excess of that 26 percent cap.
- 2. Fringe benefits are claimed using approved rates contained in the Massachusetts State-Wide Cost Allocation Plan. The following additional fixed fringe benefit charges are approved for the University:

PYE 6/30/10

Workers' Comp. Ins. .37% (S&W)
Health & Welfare(1) \$13 per week
Sick Leave Bank .20% (S&W)

- (1) Health and Welfare The State negotiated rate with collective bargaining units.
- 3. Equipment means an article of nonexpendable, tangible personal property having a useful life of more than one year, and an acquisition cost of \$5,000 or more per unit.

Jal. 9. 2009 7:29AM

INSTITUTION:

University of Massachusetts at Amherst

AGREEMENT DATE: July 8, 2009

SECTION III: GENERAL

A. LIMITATIONS:

The rates in this Agreement are subject to any statutory or administrative limitations and apply to a given grant, contract of other agreement only to the extent that funds are available. Acceptance of the rates is subject to the following conditions:

(1) Only costs incurred by the organization were included in its facilities and administrative cost pools as finally accepted costs are legal obligations of the organization and are allowable under the governing cost principles; (2) The same costs that been created as facilities and administrative costs are not claimed as direct costs; (3) Similar types of costs have been accepted consistent accounting treatment; and (4) The information provided by the organization which was used to establish the rates is lacer found to be materially incomplete or inaccurate by the Federal Government. In such situations the rate(s) would be subject to the discretion of the Federal Government.

B. ACCOUNTING CHANGES:

This Agreement is based on the accounting system purported by the organization to be in effect during the Agreement period. Of to the method of accounting for costs which affect the amount of reimbursement resulting from the use of this Agreement requirement approval of the authorized representative of the cognizant agency. Such changes include, but are not limited to, changes the charging of a particular type of cost from facilities and administrative to direct. Pailure to obtain approval may result cost disallowances.

C, FIXED RATES:

If a fixed rate is in this Agreement, it is based on an estimate of the costs for the period covered by the rate. When the act costs for this period are determined, an adjustment will be made to a rate of a future year(s) to compensate for the differenc between the costs used to establish the fixed rate and actual costs.

D. USE BY OTHER FEDERAL AGENCIES:

The rates in this Agreement were approved in accordance with the authority in Office of Management and Budget Circular A-21 Circular, and should be applied to grants, contracts and other agreements covered by this Circular, subject to any limitations above. The organization may provide copies of the Agreement to other Federal Agencies to give them early notification of the Agreement.

E. OTHER:

If any Federal contract, grant or other agreement is reimbursing facilities and administrative costs by a means other than the approved rate(s) in this Agreement, the organization should (1) credit such costs to the affected programs, and (2) apply the approved rate(s) to the appropriate base to identify the proper amount of facilities and administrative costs allocable to the programs.

BY THE INSTITUTION:	ON BEHALF OF THE FEDERAL GOVERNMENT:
University of Massachusetts at Amberst	
A YMORT OF TOTAL	DEPARTMENT OF HEALTH AND HUMAN SERVICES
(institution) (b)(6)	(D)(6)
signiture	(SIGNATURE)
\bigvee	
Joyce M. Hatch	Robert I. Aaronson
(NAME)	(NAME)
Vice Chancellor for Administration & Finance	N
(TITLE)	DIRECTOR, DIVISION OF COST ALLOCATION
	(TITLE)
7/9/2010	July 8, 2009
(DATE)	(DATE) 0742
	(2014) VIII
	Water to the contract of
	HHS REPRESENTATIVE: Michael Stanco



The Commonwealth of Massachusetts Office of the Comptroller One Ashburton Place, Room 901 Boston, Massachusetts 02108

PHONE (617) 727-5000 FAX (617) 727-2163 INTERNET: http://www.mass.gov/osc

MEMORANDUM

To:

Chief Fiscal Officers

From:

Martin J. Benison, Comptroller

Date:

June 12, 2012

Subject:

Approved FY2013 Fringe Benefit and Payroll Tax Rates

Comptroller Memo FY2013-02

Executive Summary

The purpose of this memo is to notify departments of the approved fringe benefit and payroll tax rates for FY2013. The U.S. Department of Health and Human Services has approved the fringe rate of 25.98% and a payroll tax rate of 1.29%.

Comptroller Memo FY2013-01, dated January 24, 2012, advised departments that a fringe benefit rate of 27.87% and a payroll tax rate of 1.22% had been submitted to the U.S. Department of Health and Human Services for approval. The early notice was to assist departments in planning for FY2013. The U.S. Department of Health and Human Services has approved the fringe rate of 25.98% and a payroll tax rate of 1.29%.

These rates have been calculated with the concurrence of the Secretary of the Executive Office of Administration and Finance and the U. S. Department of Health and Human Services. Please see Administrative Bulletin ANF #5.

The following components comprise the approved FY2013 fringe benefit rate:

Group Insurance	18.19%
Retirement	6.76%
Terminal Leave	<u> 1.03%</u>
Total	25.98%

This rate is applicable for both the state "5D" rate used to assess fringe benefit costs on all state funds, other than the General Fund, pursuant to M.G.L. Chapter 29, § 5D, and the "6B" rate used to assess fringe benefit costs on federally supported programs pursuant to M.G.L. Chapter 29, §6B. The rate is applied to salaries expended under object codes A01, A07, A09 and AA1 to determine these particular fringe benefit costs.

Because the costs of terminal leave salaries are allocated through the fringe benefit rate, A12, Sick-Leave Buy Back; A13, Vacation-in-Lieu; and A21, Payments for Deceased Employees object code expenditures may not also be claimed as direct costs on federally supported programs whether incurred on Federal grants, contracts or state appropriations subject to Federal reimbursement.

The following components comprise the approved FY2013 payroll tax rate:

Unemployment	0.26%
Universal Health	0.13%
Medicare Tax	0.90%
Total	1.29%

This rate is applicable to all account types pursuant to M.G.L. Chapter 151A, sections 14C and 14G for unemployment and universal health insurance, respectively, and M.G.L. Chapter 7A, sections 3, 7 and 8 for Medicare insurance. The rate is applied to regular and contract employees and is assessed to all AA and CC object codes with the exception of A75, A90, CC5, C33, C75, C90, and C98.

All fringe benefit and payroll tax assessments determined by these rates will be charged to object code D09 at the close of each accounting period.

Please note that certain expenditures made under Interdepartmental Service Agreements (ISAs) may trigger the assessment of fringe benefit and payroll tax costs to the ISA child account and both the Buyer and Seller Departments are responsible for ensuring that these amounts are adequately funded in the ISA and identified in the ISA budget.

Questions regarding this memo's rates may be directed to Taneka Simmons at (617) 973-2606.

Enc. FY2013 Fringe Agreement

Rate Summary

cc: MMARS Liaisons

Payroll Directors General Counsels Internal Distribution

Identification and Assertion of Restrictions on the Government's Use, Release, or Disclosure of Technical Data or Computer Software

The Offeror shall list all non-commercial technical data and computer software that it plans to gernerate, develop, and/or deliver in which the Government will acquire less than unlimited rights and to assert specific restrictions on those deliverables. If the Offeror lists 'NONE' or does not submit this form, the Government will assume automatically that it has "unlimited rights" to all non-commercial technical data and software generated, developed, and/or delivered.

	NON-CO	OMMERCIAL		· · · · · · · · · · · · · · · · · · ·
Technical Data or Computer Software to be Furnished With Restrictions ¹	Summary of Intended Use in the Conduct of the Research	Basis for Assertion ^{2a}	Asserted Rights Category ^{3a}	Name of Person Asserting Restrictions ⁴
(LIST)	(NARRATIVE)	(LIST)	(LIST)	(LIST)
NONE				

The Offeror shall list all commercial technical data and commercial computer software that may be included in any non-commercial deliverables contemplated under this effort, and assert any applicable restrictions on the Government's use of such commercial technical data and/or computer software. If the Offeror lists 'NONE' or does not submit this form, the Government will assume that there are no restrictions on the Government's use of such commercial items.

	COM	MERCIAL		
Technical Data or Computer Software to be Furnished With Restrictions ¹	Summary of Intended Use in the Conduct of the Research	Basis for Assertion ^{2b}	Asserted Rights Category ^{3b}	Name of Person Asserting Restrictions ⁴
(LIST)	(NARRATIVE)	(LIST)	(LIST)	(LIST)
NONE				

Date:	12/18/12
Printed Name and Title:	Carol P. Sprague, Director, Grant & Contract Admin. (b)(6)
Signature	_

Organizational Conflict of Interest Affirmations and Disclosure

Certain post-employment restrictions on former federal officers and employees may exist, including special Government employees (including but not limited to Section 207 of Title 18, United States Code, the Procurement Integrity Act, 41 U.S.C. 423, and FAR 3.104).

Without the prior approval or a waiver from the ONR, a contractor cannot simultaneously be a scientific, engineering, and technical assistance (SETA) contractor and a performer. (See Federal Acquisition Regulation (FAR) 9.503 at https://www.acquisition.gov/FAR/.) As part of the proposal submission, proposers, proposed subcontractors and consultants must affirm whether they (individuals and organizations) are providing SETA or similar support to any ONR technical office(s) through an active contract or subcontract.

Organizational Conflict of Interest as described below (if NONE so state):

Prime Contract Number	ONR Office Supported	Mitigating Action Proposed or Taken
	NONE	

Date:	12/18/12
Printed Name and Title:	Carol P. Sprague, Director, Grant and Contract Admin.
Signature	
	(End of identification and assertion)

The following ONR awards are active at UMass:

Principal Investigator	Sponsor	Award number
Vouvakis, Marinos N	Office of Naval Research (ONR)	N00014-11-1-0740
Lovley, Derek R	Office of Naval Research (ONR)	N00014-10-1-0084
Tew, Gregory N	Office of Naval Research (ONR)	N00014-10-1-0348
Bardin, Joseph C	Office of Naval Research (ONR)	N00014-12-1-0778
De Bruyn Kops, Stephen M	Office of Naval Research (ONR)	N00014-12-1-0583
Briseno, Alejandro L	Office of Naval Research (ONR)	N00014-11-1-0636
Lovley, Derek R	Office of Naval Research (ONR)	N00014-12-1-0229
Siegelmann, Hava T	Office of Naval Research (ONR)	N00014-09-1-0069
Lovley, Derek R	Office of Naval Research (ONR)	N00014-12-1-0229
Bardin, Joseph C	Office of Naval Research (ONR)	N00014-12-1-0991

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? Yes No 1.a If YES to Human Subjects
Is the Project Exempt from Federal regulations? Yes No
If yes, check appropriate exemption number.
If no, is the IRB review Pending? Yes No
IRB Approval Date:
Human Subject Assurance Number:
,
2.a. If YES to Vertebrate Animals
Is the IACUC review Pending? Yes No
IACUC Approval Date:
Animal Welfare Assurance Number
3. * Is proprietary/privileged information included in the application? Yes No
4.a. * Does this project have an actual or potential impact on the environment? Yes No
4.b. If yes, please explain:
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?
4.d. If yes, please explain:
5. * Is the research performance site designated, or eligible to be designated, as a historic place?
5.a. If yes, please explain:
6. * Does this project involve activities outside of the United States or partnerships with international collaborators?
6.a. If yes, identify countries:
6.b. Optional Explanation:
7.* Project Summary/Abstract Summary_SedimentMFC.pdf Add Attachment Delete Attachment View Attachment
8. * Project Narrative TechnicalProposal_SedimentMFC.pdf Add Attachment Delete Attachment View Attachment
9. Bibliography & References Cited References_SedimentMFC.pdf Add Attachment Delete Attachment View Attachment
10. Facilities & Other Resources Facilities-SedimentMFC.pdf Add Attachment Delete Attachment View Attachment
11. Equipment Equipment - SedimentMFC.pdf Add Attachment Delete Attachment View Attachment
12 Other Attachments Add Attachments Delete Attachments View Attachments

Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological Studies

Derek Lovley and Kelly Nevin, Department of Microbiology, University of Massachusetts

Summary

The power output of benthic microbial fuel cells needs to be increased for many desired applications. To date, the development of strategies for power optimization has been largely empirical because there is a poor understanding of the function of the current-producing cells on anode surfaces and the factors that limit their microbial growth, activity, and electron transport to the anode. Our recent studies have demonstrated that increasing the conductivity of anode biofilms can significantly increase the current densities of microbial fuel cells, and thus one aspect of the proposed research is designed to better understand the mechanisms for long-range electron transport through biofilms, as this is the one proven strategy for enhancing power output. However, it is clear that there are factors limiting the current production of the anode biofilms in marine sediments that are not being replicated in studies with pure cultures under defined conditions in the laboratory. Therefore, of equal importance is to develop an understanding of the factors controlling the activity of natural current-producing biofilms harvesting current from marine sediments. Thus, the objectives of the proposed study are to: 1) better elucidate the mechanisms for pili and biofilm conductivity and 2) determine the physiological and ecological factors controlling current production in biofilm communities in benthic microbial fuel cells. The first objective will be achieved through the design of new microbial strains, which lack cytochromes in their biofilms and along pili, as well as further studies on the impact of amino acid substitutions in PilA on pili and biofilm conductivity. For the second objective the physiological status of microorganisms in current-producing biofilms of benthic microbial fuel cells will be elucidated via meta-transcriptomic analysis. The strong possibility that protozoan grazing limits anode biofilm density, and thus current output of benthic microbial fuel cells, will also be investigated. These studies on the mechanisms for the metallic-like conductivity of pili and current-producing biofilms will provide basic information needed for the better design of microorganisms with enhanced current-producing capabilities, as well as contribute to the new field of conducting biological materials that has emerged from this research. The studies on gene expression and protozoan grazing are expected to finally answer the key question of why benthic microbial fuel cells have lower power outputs than their laboratory analogs.

References

- 1. Ahuja, U., P. Kjelgaard, B. L. Schulz, L. Thony-Meyer, and L. Hederstedt. 2009. Haem-delivery proteins in cytochrome c maturation system II. Mol. Microbiol. 73:1058-1071.
- 2. Badawi, N., A. R. Johnsen, K. K. Brandt, J. Sørensen, and J. Aamand. 2012. Protozoan predation in soil slurries compromises determination of contaminant mineralization potential. Environ. Poll. 170:32-38.
- 3. Craig, L., M. E. Piquie, and J. A. Tainer. 2004. Type IV pilus structure and bacterial pathogenicity. Nature Reviews Microbiology 2:363-378.
- 4. Elifantz, H., L. A. N'Guessan, P. J. Mouser, K. H. Williams, M. J. Wilkins, D. E. Holmes, C. Risso, P. E. Long, and D. R. Lovley. 2010. Expression of acetate permease- like genes in subsurface communities of *Geobacter* species under fluctuating acetate conditions. Microb Ecol 73:441-449.
- 5. Fenchel, T. 1969. The ecology of the microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna commutates with special reference to the ciliated protozoa. Ophelia 6:1-182.
- 6. Franks, A. E., R. H. Glaven, and D. R. Lovley. 2012. Real-time spatial gene expression analysis within current-producing biofilms. ChemSusChem 5:1092-1098.
- 7. Franks, A. E., K. P. Nevin, R. H. Glaven, and D. R. Lovley. 2010. Microtoming coupled to microarray analysis to evaluate the spatial metabolic status of *Geobacter sulfurreducens* biofilms. ISME Journal 4:509-519.
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EQUIPMENTUniversity of Massachusetts, Amherst

Laboratory Equipment includes:

Shimadzu LCMS-2020 mass spectrometer with electrospray interface for high speed scanning and high sensitivity applications; Applied Biosystems 3730XL DNA analyzer; Axon Instruments Genepix 4000B Microarray Scanner; Genpix software and Acuity database and data handler; Applied Biosystems 7500 RT-PCR; BioRad Experion Automated Electrophoresis System; Varian Cary 50 Bio UV/Vis spectrophotometer; Shimadzu UV-2401PC UV/VIS spectrophotometer; Hewlett Packard (HP) HP6890 capillary gas chromatograph (TCD/FID/ECD detectors); Perkin Elmer Clarus 600 capillary (FID) gas chromatograph with turbomatrix headspace analyzer and autosampler; Shimadzu GC-8A/INUS gas proportional counter; Shimadzu GC-8 gas chromatograph; HP series 1100 HPLC with diode array fluorescence detectors and autosampler; Shimadzu SPD10, CMB-20A, and SPD6A HPLCs with UV/IR detectors and autosamplers; Shimadzu OP-2010Ultra GCMS system; Chemchek Instruments Kinetic Phosphorescence Analyzer KPA-11 and autosampler; Trace Analytical reduction gas analyzer for H₂ measurements; multiple Gamry multichannel and Amel single channel potentiostats with electrochemical software; Sulfide Gas Minisensor, Redox Mini Electrode, Dionex ion chromatography system ICS-1000 with degas chromeleon SE and autosampler; Dionex system DX 500; Nikon Eclipse E600 epifluorescent microscope with Hamamtsu Digital CCU camera; Nikon E400 phase contrast microscope with SPOT RT900 SE monochrome digital camera and OED image software; Leica TCS SP5 Spectral Confocal Upright Microscope with scanning stage and fluorescence/reflection detectors; Amersham Pharmacia fast protein liquid chromatography system; Amersham Multiphor II 2-D electrophoresis system; multiple spectrophotometers suitable for scans and kinetic studies; BioRad flourometer; MP Biomedical FastPrep homogenizer; multiple low speed, ultra, and micro-centrifuges; electrophoresis equipment for agarose gels and polyacrylamide gels; liquid scintillation counter; 7-Coy anaerobic chambers; anaerobic gassing apparatus; incubators; Baker laminar flow sterile UV hoods; multiple BioRad, Eppendorf, and MJ Research thermocyclers; hybridization ovens; UV cross linkers; UV light boxes; multiple electroporation apparatus; multiple blotting apparatus; french press; sonicator; speed vacuum system; photographic equipment; walk in incubators for sediment and cultures; -80 °C freezers; Milli-Q and Nanopure deionized water filtration units; Anprolene ethylene oxide sterilizer with scrubber; water baths; pipettors; refrigerators; etc.

Laboratories are equipped with fume hoods and gas, steam and distilled water lines. Additional autoclaves, walk-in incubators, low speed refrigerated centrifuges, ultracentrifuges, and rotors are available in the Department of Microbiology.

Computer equipment includes:

Sun Fire V880 server, CDC 2460 Simpheny-DB 2460 Dual Intel PIV Xeon Server; NIXSYS NIX2000-8RD Tyan Thunder 2xAMD Opteron dual core with RAID

Mac or PC workstations for each postdoc, graduate student, and for analytical equipment.

FACILITIES AND OTHER RESOURCES University of Massachusetts, Amherst

Dr. Lovley's laboratory in the Department of Microbiology:

Laboratory encompasses 14,336 square feet, of which 11,600 square feet has been recently constructed. The laboratory is fully equipped for investigations on the physiology, ecology and molecular biology of anaerobic microorganisms.

The following additional facilities are available for analysis:

Electron Microscopy Facilities in the departments of Microbiology, Polymer Science and Physics at Umass Amherst, MALDI-TOF/MS analyses at the University of Mass Worcester, Multiplex DNA Sequencing analyses at the University of Mass Worcester

University of Massachusetts Facilities and Equipment Safety:

At the University of Massachusetts, Amherst, a university wide safety plan is in effect through the Environmental Health and Safety Program. This plan is based on applicable health and safety standards promulgated by Federal and State agencies including OSHA Occupational Exposure to Hazardous Chemicals in Laboratories and published standards of nationally recognized professional health and safety groups. In accordance with federal mandates the following committees are established at the University of Massachusetts: the Radioisotope Use Committee, the Recombinant DNA Committee (Guidelines for Research involving recombinant DNA molecules by the NIH followed), Biological Hazards Committee, Institutional Animal Care and Use Committee and Chemical Hazards Committee. These committees have established safety and health policies in accordance with federal, state, and local laws and regulations. Our laboratory is regularly inspected for compliance to health and safety as well as waste minimization and waste disposal requirements.

Technical Proposal Cover Page

ONR BAA Announcement #13-001

Title: Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological Studies

Prime Offeror: University of Massachusetts, Amherst

Principal Investigator: Derek R. Lovley

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Proposed period of performance: Three Years (3/1/13 to 2/29/16)

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Technical Approach

Introduction

The Office of Naval Research has expressed interest in microbial fuel cells because they have the potential to power electronic devices at the bottom of the sea, represent non-explosive, non-toxic power sources that can potentially be incorporated into monitoring devices, and have substantial potential to stimulate the degradation of organic contaminants in marine sediments. Optimizing these applications requires an understanding of how microorganisms transfer electrons onto the anode surfaces of microbial fuel cells and the factors controlling the rate and extent of this electron transfer, not only in the laboratory, but also in marine sediments. Furthermore, the recent discovery of metallic-like conductivity in current-producing biofilms and the pili networks that course through these biofilms has opened a new chapter in biological electron transport that should be pursued for its intrinsic basic science importance, as well as for the many applications that are likely to emerge from this fundamentally new concept for electron transport through biological materials.

Although there is strong evidence for the metallic-like conductivity of biofilms and pili networks for *Geobacter sulfurreducens* (20, 24), this concept is revolutionary. "Extraordinary claims require extraordinary evidence" and there has been substantial debate whether the pili of *G. sulfurreducens* have metallic-like conductivity and whether metallic-like conductivity is an important feature controlling the output of microbial fuel cells. Resolution of this debate seems possible with a few additional, well-designed studies that will clear up any ambiguities.

At the same time that the understanding of the fundamentals of electron transport through current-producing biofilms has advanced dramatically, there have been essentially no new insights into the microbiology of the natural current-producing biofilms that colonize anodes harvesting current from marine sediments for nearly a decade. As outlined below, the technology is now available to diagnose the physiological status of the microorganisms in these natural biofilms in order to determine what factors might be limiting their rates of current production. Furthermore, it seems likely that protozoan grazing, recently recognized as an important factor culling *Geobacter* communities during groundwater bioremediation, may substantially limit anode biofilm growth and thus current output.

Therefore the one of the two of the objectives of the proposed research is to perform new studies designed to evaluate pili and biofilm conductivity with approaches that should alleviate concerns with the methods initially employed in the studies which first revealed apparent metallic-like conductivity. The second objective is to examine the *in situ* physiology and ecology of anode biofilms in marine sediments to better understand the factors that might be limiting the current output of benthic microbial fuel cells.

BACKGROUND

Our most recent research on microbial fuel cells was designed to quantify electronic properties of *Geobacter sulfurreducens* biofilms, such as conductivity and capacitance, and determine the role of various components of cells in contributing to these electronic properties in order to gain insight into the factors controlling current production. *G. sulfurreducens* was the organism of choice in these initial studies because: 1) it is closely related to the *Geobacteraceae* that typically predominant on anodes harvesting electricity from sediments or wastewater; 2) *G. sulfurreducens* produces the highest current density of any known pure culture; and 3) a genetic

system and a genome-scale metabolic model are available and a host of omics tools have been perfected for the study of this organism.

Metallic-like Conductivity of Pili

One of the most practical benefits of this foundational research was the demonstration that biofilm conductivity is a decisive variable in controlling the current output of microbial fuel cells (22). Strains engineered to produced more pili had more conductive biofilms and produced higher current densities. These results represent the first example in which an understanding of microbial physiology has led to a predictive model that could directly lead to improved current production in microbial cells.

This practical result and other important findings stem from the discovery that the pili and biofilms of *G. sulfurreducens* have metallic-like conductivity (24). This finding is exciting at multiple levels. Most basically, metallic-like conductivity has not been previously described in biological materials, thus this discovery is a paradigm shift in long- range biological electron transport. Although it had previously been speculated that the pili of *G. sulfurreducens* were conductive along their length (36) and could form conductive networks in biofilms (37), there was substantial skepticism because there was no known mechanism for conduction along a biological protein filament, and it was generally regarded that biofilms acted as insulators, not conductors. The discovery that *G. sulfurreducens* biofilms have metallic-like conductivity explains how *G. sulfurreducens* can form thick biofilms in which even cells at great distance from the anode are metabolically active and contribute to current production. This conductivity is key to the production of high current densities. It is also expected that the metallic-like conductivity of pili and/or biofilms will have novel applications in the emerging field of bioelectronics.

Multiple lines of evidence indicated that conduction along the length of the pili was metallic-like (20, 24). The first hint was from studies that examined conductance as a function of temperature. As the temperature was dropped from room temperature there was an initial exponential increase in conductivity that continued through the physiological range of temperatures examined (i.e. 0-27 °C). In contrast, a decrease in conductivity as temperature is lowered is expected via the traditional mode of biological electron transfer in which electrons hop or tunnel between discrete redox-active electron carriers. The temperature dependence of the pili conductivity was similar to that of polyaniline, a synthetic organic polymer with metallic-like conductivity.

Further evidence for metallic-like conductivity included a dramatic increase in pili conductivity with decreasing pH, consistent with the increase in conductivity that is observed when synthetic conducting polymers are doped with protons. The highest pili conductivity observed was at pH 2, the lowest pH tested. Redox active electron carriers, such as *c*-type cytochromes are denatured at this low pH at which conductivity was at a maximum. Furthermore, there is no known model in which lowering the pH should dramatically increase the rate of electron transfer via hopping/tunneling. Pili also exhibited a very large magnetoconductance response, consistent with metallic-like conductivity and inconsistent with electron hopping/tunneling.

Although we had previously discovered that there were cytochromes associated with the pili, atomic force microscopy of untreated pili still attached to cells demonstrated that as previously predicted (15), the cytochromes were spaced much too far apart for electron hopping/tunneling along the pili to be feasible (23). Furthermore, denaturing cytochromes in pili preparations had no impact on pili conductivity (24).

Metallic-Like Conductivity in Biofilms Attributed to Pili Networks

Current-producing biofilms of *G. sulfurreducens* also had substantial conductivity (24). Temperature-dependence studies indicated that the biofilm conductivity was also metallic-like. There was a strong correspondence between biofilm conductivity and the abundance of PilA, the pili monomer, in *G. sulfurreducens* biofilms. Furthermore, genetically engineering a strain of *G. sulfurreducens* to produce more pili greatly increased biofilm conductivity and current production (Leang, manuscript in preparation). In contrast, there was a negative correlation between the abundance of *c*-type cytochromes, the hypothesized agents for electron hopping/tunneling through biofilms, and conductivity (23). Genetically engineering strains for lower cytochrome abundance yielded strains with increased conductivity rather than the lower conductivity that would be expected if electron hopping/tunneling was the mode of electron conductance through the biofilms.

Mechanisms for Metallic-Like Conductivity

Preliminary structural studies of purified pili provided evidence for overlapping pi-pi orbitals, which are known to confer metallic-like conductivity to synthetic organic conducting polymers (24). Thus, it was hypothesized that there were specific aromatic amino acids in the PilA structure that contributed overlapping pi-pi orbitals, which accounted for the metallic-like electron conduction along the length of *G. sulfurreducens* pili. It seemed most likely that these aromatic amino acids were localized in the carboxyl end of the peptide because the carboxyl portion of *G. sulfurreducens* differs significantly from the PilA of other organisms and because preliminary structural modeling predicted that the carboxyl aromatic amino acids would be exposed on the outer surface of the filaments.

(b) (4)

(b) (4)

For example, direct interspecies electron transfer to G. sulfurreducens from G. metallireducens requires that G. sulfurreducens have pili and OmcS (39). This has been attributed to OmcS providing an electrical connection with G. metallireducens with long-range electron transport along the pili (17). (b) (4)

In contrast, cells multiple cell lengths away from the anode actively contribute to current production in wild- type biofilms (6, 7). (b) (4)

Demonstrating the Importance of Biofilm Conductivity for Power Output of Microbial Fuel Cells

There was considerable confusion in the literature about the importance of biofilm
conductivity in contributing to the current density of microbial fuel cells. This could be attributed
in part to the fact that no previous studies, other than ours, had directly measured biofilm
conductivity. We demonstrated that there was a strong, direct correlation between biofilm
conductivity and current density (22). Higher conductivities also contributed to lower charge
transfer resistances at the biofilm-anode interface. Higher conductivities could clearly be
attributed to higher pili abundances, consistent with our hypothesis that a network of conductive
pili is responsible for long-range electron transport through the biofilms.

Evaluation of the Hypothesis that Cytochromes Account for Pili and Biofilm Conductivity The finding of metallic-like conductivity in pili and biofilms of G. sulfurreducens was unexpected. Many previous studies had suggested a more traditional form of biological electron transfer in which electrons hop/tunnel between the abundant c-type cytochromes of G. sulfurreducens. Therefore, this alternative hypothesis that electron hoping/tunneling is responsible for the conductivity of G. sulfurreducens biofilms was also investigated in detail. Multiple lines of evidence, derived from studies which relied on different basic assumptions, refuted the cytochrome hypothesis (23). For example, measurements of the heme content of biofilms demonstrated that there were not enough cytochromes present to account for electron conduction through the biofilms. Furthermore, there was no correlation between biofilm conductivity and cytochrome content. In fact, reducing cytochrome abundance by deleting genes for cytochrome production actually increased biofilm conductivity and power output. Denaturing cytochromes in the biofilms had no impact on conductivity. Biofilm conductivity did not show a redox peak, as would be expected for conduction via redox carriers (23). Although our previous research had demonstrated the presence of cytochromes on pili, examination of the pili with atomic force microscopy demonstrated that the cytochromes were spaced too far apart for electron hopping/tunneling between cytochromes to account for the electron conduction along the pili (23). These findings were consistent with multiple previous lines of evidence for metallic-like conductivity and inconsistent with the electron hopping/tunneling hypothesis.

Supercapacitor Properties of Geobacter sulfurreducens biofilms

Studies involving electrochemical impedance spectroscopy, cyclic voltammetry and charge-discharge cycling demonstrated that *G. sulfurreducens* biofilms function as supercapacitors (21). The biofilms also have low self-discharge and good charge/discharge reversibility. The superior electrochemical performance of the biofilm could be related to its high abundance of cytochromes, providing large electron storage capacity, its nanostructured network with metallic-like conductivity, and its porous architecture with hydrous nature, offering

prospects for future low cost and environmentally sustainable energy storage devices. This discovery was the cover image for the issue of ChemSusChem in which it was published.

Real-Time Imaging of Gene Expression in Current-Producing Biofilms

The physiological status of cells within current-producing biofilms is a key parameter controlling current production. The different models for current production in G. sulfurreducens biofilms make significantly different predictions about how cells in different layers of the biofilm should be metabolizing. In order to obtain data on the metabolism of cells in current- producing biofilms, a strategy was developed for analyzing gene expression within Geobacter sulfurreducens anode biofilms in real time with short half-like fluorescent protein reporters (6). Proof of concept studies with a reporter for *nifD*, which encodes one of the genes for nitrogen fixation, demonstrated that the reporter could readily detect expression of *nifD* when ammonium was excluded from the medium. Repression of nifD expression following the addition of ammonium was readily apparent from the loss of reporter signal. In a similar manner, the gene for the key TCA cycle enzyme, citrate synthase, was highly expressed when acetate was supplied and current levels were high. However, expression was repressed when the anode biofilms were deprived of acetate. Monitoring the expression of several metabolic genes suggested that cells throughout the anode biofilm were metabolically active and contributing to current production. This finding lends more support to the concept of long-range electron transfer through anode biofilms.

Conductivity of Mixed Species Biofilms

In order to determine the environmental relevance of the conductivity observed in *G. sulfurreducens* biofilms, the conductivity of 'natural' biofilms that grew on electrodes from an environmental inoculum was measured (19). Biofilm conductivities were high despite the fact that more than 50% of the microorganisms in the biofilm were not *Geobacter* species. These results demonstrate the significance of conductive biofilms in harvesting electricity from environmental sources.

Conductivity of Reduced Marine Sediments

It has been proposed (32) on the basis of indirect evidence that soils and sediments may be conductive in a manner similar to that of anode biofilms. (b) (4)

Objectives and Hypotheses

There are two main objectives in the proposed research. The first objective is designed to better understand the mechanisms for pili and biofilm conductivity with approaches designed to unequivocally resolve whether metallic-like conductivity can account for long-range electron transport. The second objective is designed to initiate a new line of research studying natural current-producing biofilms harvesting current from marine sediments in order to determine the physiological and ecological factors controlling current production in biofilm communities in benthic microbial fuel cells.

Objective 1. Determine the mechanisms for pili and biofilm conductivity by measuring the conductivity of microbial strains that produce pili that are not contaminated with cytochromes and by determining which amino acids in PilA are required for pili and biofilm conductivity.

Hypotheses

- 1. Strains of *Geobacter sulfurreducens* that are unable to produce cytochromes will produce pili and biofilms that are conductive.
- 2. Expression of the *Geobacter sulfurreducens*' gene for PilA in *Pseudomonas aeruginosa* will yield a *P. aeruginosa* strain that will produce conductive pili and biofilms.
- 3. All five of the aromatic amino acids in the carboxyl end of PilA are required for maximum pili conductivity and conductive biofilm function.

Objective 2. Determine physiological and ecological factors controlling current production in biofilm communities in benthic microbial fuel cells.

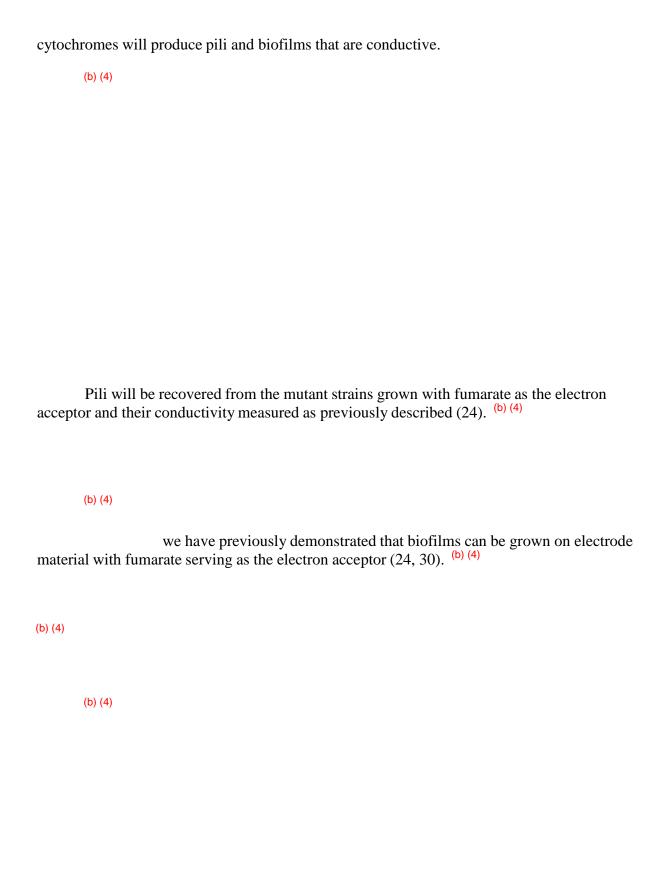
Hypotheses

- 1. Meta-transcriptomic analysis of the biofilms of benthic microbial fuel cells will reveal physiological stresses in the anode community limiting current production.
- 2. Protozoan grazing limits anode biofilm growth and current output of benthic microbial fuel cells.

Approach

The studies are designed to address the key remaining questions about the basic mechanisms of electron transfer along pili and through biofilms in defined laboratory current-producing systems, as well as to begin transferring mechanistic insights and methods developed in the study of laboratory systems to more directly examine the functioning of the more complex biofilms associated with the anode of benthic microbial fuel cells deployed in sediments. Studies in defined systems will continue to focus on *Geobacter sulfurreducens*. Studies on the microbiology of benthic fuel cell anodes will use meta-transcriptomic methods that we have recently developed for the study of other microbial communities to assess the physiological status of the microbes in anode biofilms under different conditions. Furthermore, the possibility that protozoan grazing is a major factor limiting current production in benthic marine fuel cells will be addressed.

Objective 1. Hypotheses 1. Strains of Geobacter sulfurreducens that are unable to produce



Purified OmcS will be obtained as previously described (35).

Objective 2. Hypothesis 1. Meta-transcriptomic analysis of the biofilms of benthic microbial fuel cells will reveal physiological stresses in the anode community limiting current production.

Benthic microbial fuel cells produce less current than laboratory microbial fuel cells even when the anodes inserted into the sediments are pre-coated with biofilms capable of high current production and the biofilms are provided with high concentrations of electron donor. This suggests that there are important factors limiting current output in sediments that are not replicated in defined laboratory systems.

One likely explanation is that the current-producing cells in anode biofilms of benthic fuel cells are under as-yet-unknown physiological stresses that are limiting their growth and activity. Previous studies on the optimization of *in situ* groundwater bioremediation demonstrated that analyzing the *in situ* gene expression of the subsurface community can provide important insights into rates of microbial activity, nutrient limitations, and other environmental conditions deleterious to microbial growth (4, 11-14, 26-28, 33). These initial studies targeted expression of specific genes. However, recent advances in DNA sequencing technology has made it possible to broadly examine gene expression in natural microbial communities.

For example, our laboratory has been studying the function of anaerobic digesters converting waste organic matter to methane and we have successfully used meta-transcriptomic analysis of these complex communities to elucidate mechanisms for interspecies electron transfer. (b) (4)

Our significant experience in studying gene expression in *Geobacter* species under diverse conditions will aid in interpreting the gene transcript patterns. For example, it is possible to estimate growth rates of the cells from the relative abundance of ribosomal protein gene transcripts (10), as well as diagnose nutrient limitations and other physiological stresses (4, 12-14, 26-28, 33). Thus, it should be feasible to determine the physiological state of the cells in the biofilms and what factors are likely to be limiting activity.

These analyses should answer key questions, such as whether electron donor availability is the overarching factor limiting power output. Analysis of gene expression on anodes incubated for various lengths of time will provide insight into factors that begin to limit power production in longer-term incubations.

Initially whole biofilms will be analyzed. However, as we have previously demonstrated, it is possible to measure gene expression at different depths of biofilms (7), providing insight into biofilm metabolic heterogeneities and which layers of the biofilm are contributing to current production.

Although sophisticated physiological analyses and gene expression studies have been conducted on current-producing biofilms under highly defined conditions with pure cultures (9, 30), studies on the mixed communities harvesting current in sediments have been restricted to simple identification of the phylogenetic affiliation of the cells within the biofilms. Therefore, these transcriptomic studies are expected to provide unprecedented insights the physiological status of the microorganisms producing current in benthic microbial fuel cells and the factors that might be limiting their performance.

Objective 2. Hypothesis 2. Protozoan grazing limits anode biofilm growth and current output of benthic microbial fuel cells.

Another major difference between benthic microbial fuel cells and pure-culture laboratory systems is the potential presence of bacterial predators in the natural environment. Anaerobic protozoa are abundant in anaerobic marine sediments (5) and our recent research in subsurface bioremediation has demonstrated that stimulating the growth of *Geobacter* species in the subsurface results in a dramatic response of specific protozoa which feed on *Geobacter* and reduce *Geobacter* populations (Holmes et al., manuscript submitted). Therefore, it seems likely that the anode biofilms of benthic microbial fuel cells would provide an excellent 'dinner plate' for protozoa feeding on *Geobacteraceae* and other microorganisms in the biofilm and that this protozoan grazing could limit the thickness of the anode biofilm and hence power output of benthic microbial fuel cells.

This hypothesis will be examined with several approaches. First, it will be determined whether there is an enrichment of specific protozoa associated with anode biofilms harvesting current from marine sediments. Anode biofilm samples will be obtained from laboratory incubations of benthic fuel cells in marine sediments routinely carried out in our laboratory as well as samples that will be obtained from collaboration with researchers at SPAWAR in San Diego. Biofilms will be scraped from the anodes and DNA extracted with methods routinely in use in our laboratory. Sequences of 18S rRNA will be amplified with primers in routine use in our laboratory for recovering these eukaryotic sequences to determine which protozoa are associated with the biofilms and their relative abundance. These results will be compared with control biofilms that grow on electrode materials inserted in the sediments, but not connected to a cathode. The numbers of protozoa specifically enriched in current-harvesting electrodes will be

further quantified with quantitative PCR, using methods that we have developed for tracking protozoa populations in subsurface environments. A specific enrichment of one or more genera of protozoa in current-harvesting biofilms will indicate that protozoa are responding to the presence of the anode biofilm.

If, as expected, there is an enrichment of protozoa associated with the anode biofilm, then the impact of these protozoa on current production will be further investigated with a combination of studies with pure culture anode biofilms as well as natural, mixed community biofilms. For example, we have established cultures of the protozoan *Breviata anathema*, which is closely related to the protozoa that bloom during enhanced *Geobacter* growth in the subsurface, and can be maintained in the laboratory on a diet of *Geobacter* cells. We will inoculate *B. anathema* into laboratory microbial fuel cells in which the anode is colonized by *G. sulfurreducens*. If protozoan grazing has the potential to significantly effect current production, then it is expected that the presence of *B. anathema* will greatly reduce the power output of the fuel cells as the protozoa graze the biofilm.

Studies will also be conducted with anodes incubated in marine sediments. There are several strategies that can be used to diminish protozoan activity in the sediments. These include the addition of cyclohexamide, a eukaryotic antibiotic that will kill protozoa, but not bacteria (2, 34). Another possibility is heat-shock. Short-term heating to 45 °C kills protozoa, while having little impact on the function of sediment bacteria once temperatures are returned to *in situ* levels (40). If protozoa are an important factor limiting current production then these treatments are expected to increase the current output of benthic fuel cells. If, as expected, protozoa are found to have an important impact on current output, anode designs can be modified to exclude protozoa grazing.

Benefits and Significance

These studies will provide important insights into the functioning of benthic microbial fuel cells and the factors limiting current output, as well as further elucidating the mechanisms for electron transport through conductive biofilms. Both types of studies are expected to lead to improved design of benthic microbial fuel cells. To date, the only predictive model that has led to the design of microorganisms with enhanced power output is the model of metallic-like conductivity via pili networks. Therefore, it is important to better understand how this long-range, metallic-like conductivity works. Elucidating the mechanisms for metallic-like conductivity will also greatly advance this exciting new paradigm shift in biological electron transport and provide information necessary for the design of improved sensors and biologically conductive materials (16). Furthermore, a better understanding of microbe-electrode electron exchange is expected to aid in the understanding of direct interspecies electron transfer (39), and optimization of bioenergy strategies, such as microbial electrosynthesis (18, 29, 31) and conversion of wastes to methane (17, 25).

Although there have been significant advances in the understanding how pure culture, current-producing biofilms function, there has been a severe lack of analysis of the functioning of the biofilms associated with actual benthic microbial fuel cells. The studies outlined here on the meta-transcriptomics of benthic fuel cell anode biofilms will provide the first insights into the physiological status of these biofilms and the factors likely to be limiting current production.

The studies on protozoan grazing are expected to identify another key factor limiting current production and solve, at least in part, the mystery of why power output in marine benthic fuel cells is so much lower than those in the laboratory.

Project Schedule and Milestones

Tasks to be completed in each year are as follows:
Year 1
(b) (4)
Evaluate possibility for <i>in vitro</i> assembly of PilA subunits.
(b) (4)
Begin incubation of sediment microbial fuel cells for metatranscriptomic and protozoan studies.
Year 2
Measure conductivity of individual pili (b) (4) with electrostatic force microscopy.
(b) (4)
Further evaluate possibility for <i>in vitro</i> assembly of PilA subunits.
Further evaluate possibility for <i>in vitro</i> assembly of PilA subunits. (b) (4)

Metatranscriptomic sampling and preliminary analysis of anode biofilms in a time series

of sediment microbial fuel cells.

Sampling of anode biofilms for 18S rRNA sequence analysis of protozoan community.

Year 3

Determine conductive properties of pili produced from *in vitro* assembly of PilA subunits.

(b) (4)

Full analysis of anode metatranscriptomic studies complete and physiological stresses in anode biofilm identified.

Evaluate role of protozoa in reducing current through grazing with pure cutlures and protozoa inhibition studies in sediments.

Reports

The following reports will be prepared:

Technical and financial reports as specified in Office of Naval Research Reporting Requirement

Final Technical and Financial Report

Peer-reviewed publications

Management Approach

Derek Lovley, the principle investigator will be responsible for the overall grant management. Research directions will set and overseen by the principal investigator, and co-principle investigator, Kelly Nevin, with Lovley primarily overseeing genetic manipulation and biochemical studies and and Nevin overseeing growth of microorganisms in electrochemical systems, as well as bioelectrochemical and biophysical investigations. The principal and co-principle investigators and postdoctoral researcher will have formal weekly meetings to discuss research progress and coordinate future work. More frequent, informal meetings on a daily basis are expected.

Current Support

Derek R. Lovley, University of Massachusetts

Electrofuels via Direct Electron Transfer from Electrodes to Microbes II
U.S Department of Energy: Advanced Research Projects Agency - Energy
Lawrence 2011 - Lyne 2012

January 2011 – June 2013

3 months effort per year

\$3,026,719

Mechanisms for Electron Transfer through Electrochemically Active Biofilms

Office of Naval Research

January 2010 – December 2012

0.75 month effort per year

\$621,508

Coupled *In Silico* Microbial and Geochemical Reactive Transport Models: Extension to Multi-Organism Communities, Upscaling and Experimental Validation

U.S Department of Energy

May 2010 – May 2013

0.5 months effort per year

\$629,381

Mechanisms for Electron Transfer Through Pili to Fe(III) Oxide in Geobacter

U.S Department of Energy

June 2010 – May 2013

0.5 months effort per year

\$814,534

Systems Level Analysis of the Function and Adaptive Responses of Methanogenic Consortia

U.S Department of Energy

July 2010 – June 2013

1.5 months effort per year

\$1,220,828

Real Time Monitoring of Rates of Subsurface Microbial Activity Associated with Natural Attenuation and Electron Donor Availability for Engineered Bioremediation with Current-Producing Microorganisms

U.S Department of Energy

September 2011 – August 2014

0.5 months effort per year

\$1,001,671

Mechanisms Underlying the Metallic-Like Conductivity of Microbial Nanowires

Office of Naval Research

January 2012 - December 2014

0.75 months effort per year

\$638,349

Pending Support:

Rewiring a Microbial Chassis to Optimize Electrosynthesis

Office of Naval Research

4 years

0.75 month effort per year

\$1,598,052

This proposal:

Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological Studies

Office of Naval Research

3 years

0.5 month effort per year

\$605,513

Current Support

Kelly Nevin, University of Massachusetts

Electrofuels via Direct Electron Transfer from Electrodes to Microbes II U.S Department of Energy: Advanced Research Projects Agency - Energy January 2011 – June 2013 4 months effort per year as Co-PI

\$3,026,719

Mechanisms for Electron Transfer through Electrochemically Active Biofilms

Office of Naval Research

Jan 2010 - Dec 2012 4 months effort per year as Co-PI \$621,508

Real Time Monitoring of Rates of Subsurface Microbial Activity Associated with Natural Attenuation and Electron Donor Availability for Engineered Bioremediation with Current-Producing Microorganisms

U.S Department of Energy

September 2011 – August 2014 3 months effort per year as Co-PI \$1,001,671

Pending Support:

Rewiring a Microbial Chassis to Optimize Electrosynthesis

Office of Naval Research

4 months effort per year as Co-PI 4 years

\$1,598,052

This proposal:

Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological **Studies**

Office of Naval Research

3 years 4 months effort per year as Co-PI \$605,513

Biographical Sketch-Derek R. Lovley

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EDUCATION:

University of Connecticut	B.A.	1971-1975	Biological Sciences
Clark University	M.A.	1976-1978	Biological Sciences
Michigan State University	Ph.D.	1978-1982	Microbiology
Virginia Polytechnic Institute	Postdoctora	al 1982-1984	Microbiology

PROFESSIONAL APPOINTMENTS:

1999-Present: Distinguished University Professor, University of Massachusetts 2004-Present: Associate Dean, College of Natural Resources and the Environment

1997-2004: Department Head, Department of Microbiology

1995-1999: Professor, Department of Microbiology, University of Massachusetts

1984-1995: Research Hydrologist (GS-15), Water Resources Division, U.S. Geol. Survey

SELECT AWARDS:

2003-Present:	Most Highly Cited, Institute for Scientific Information (ISI H factor: 100)
2009:	Top 50 Invention for 2009, Time Magazine
2007:	Life Achievement Award, AHES Foundation
2007	'Top Cited Author', Environmental Microbiology
2006	Top contributors to biotechnology in the last decade, <i>Nature Biotechnology</i>
2004	Proctor and Gamble Award in Applied and Environmental Microbiology
2003	Time Magazine featured Environmental Innovator
1997	Fellow, American Academy of Microbiology
1992	Grand Winner, <i>Popular Science</i> , Best of What's New in Environmental
	Technology

RELEVANT PATENTS:

Microbial Production of Multi-Carbon Chemicals and Fuels from Water and Carbon Dioxide using Electric Current

Geobacteraceae Strains and Methods (patent application on genetic modification for higher current production)

Systems and Methods for Microbial Reductive Dechlorination of Environmental Contaminants (patent on use of cathode to promote reductive dechlorination)

Microbial fuel cells (joint patent with Toyota)

Microbial Nanowires Related Systems and Methods of Fabrication

SELECTED RELEVANT PUBLICATIONS (Microbe-Electrode Interactions 2010-2012)

Lovley D.R. and K.P. Nevin. 2013. Electrobiocommodities: Powering microbial production of fuels and commodity chemicals from carbon dioxide with electricity. Curr. Opin. Botechnol. (in press).

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- Liu, F., A.-E. Rotaru, P. M. Shrestha, N. S. Malvankar, K. P. Nevin, and D. R. Lovley. 2012. Promoting direct interspecies electron transfer with activated carbon. Energy. Environ. Sci. 5:8982-8989.
- Zhang T, Nie H, Bain TS, Lu H, Cui M, Snoeyenbos-West OL, Franks AE, Nevin KP, Russell TP, Lovley DR. 2012. Improved cathode materials for microbial electrosynthesis. Energy. Environ. Sci. 5:DOI:10.1039/C1032EE23350A
- Malvankar, N. S., and D. R. Lovley. 2012. Microbial nanowires: a new paradigm for biological electron transfer and bioelectronics. ChemSusChem 5:1039-1046.
- Rotaru, A.-E., P. M. Shrestha, A. Liu, T. Ueki, K. P. Nevin, and D. R. Lovley. 2012. Interspecies electron transfer via H2 and formate rather than direct electrical connections in cocultures of Pelobacter carbinolicus and Geobacter sulfurreducens. Appl. Environ. Microbiol. 78: 7645-7651.
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- Shrestha, P. M., A.-E. Rotaru, Z. M. Summers, M. Shrestha, F. Liu, and D. R. Lovley. 2012. Transcriptomic and genetic analysis of direct interspecies electron transfer. (manuscript submitted).
- Smith, J. A., D. R. Lovley, and P. L. Tremblay. 2012. Outer cell surface components essential for Fe(III) oxide reduction by Geobacter metallireducens. Appl Environ Microbiol (in press).
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EDUCATION AND TRAINING

B.S., Biology, Rensselear Polytechnic Institute
 Ph.D., Microbiology University of Massachusetts Amherst
 Post-Doctoral Research Associate with Dr. Derek R. Lovley, Department of Microbiology, University of Massachusetts Amherst
 Research Assistant Professor, Department of Microbiology, University of Massachusetts Amherst

2012-present Research Associate Professor, Department of Microbiology, University of Massachusetts Amherst

RELATED RESEARCH ARTICLES

- Nevin, K.P., T.L. Woodard, A.E. Franks, Z.M. Summers, and D.R. Lovley. 2010. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. mBio, doi:10.1128/mBio.00103-10.
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- Ueki T, Nevin KP, Leang C, Lovley DR. 2013. Deletion of a hydrogenase required for growth of Clostridium ljungdahlii on hydrogen provides evidence for direct electron transfer during microbial electrosynthesis. (manuscript submitted)
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RELATED PATENTS:

- Microbial Production of Multi-Carbon Chemicals and Fuels from Water and Carbon Dioxide using Electric Current
- Geobacteraceae Strains and Methods (patent application on genetic modification for higher current production)
- Microbial fuel cells (joint patent with Toyota)

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RESEARCH & RELATED Senior/Key Person Profile

PROFILE - Project Director/Principal Inves	stigator		
Prefix: Dr . * First Name: Derek	Middle Name:		
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PROFILE - Senior/Key Person 1			
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Position/Title: Associate Professor Department:	Microbiology		
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*Attach Biographical Sketch Nevin-Biosketch-SedimentMFC.p Add A	Attachment Delete Attachment View Attachment		
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ADDITIONAL SENIOR/KEY PERSON PROFILE(S)	Add Attachment Delete Attachment View Attachment		
Additional Biographical Sketch(es) (Senior/Key Person)	Add Attachment Delete Attachment View Attachment		
Additional Current and Pending Support(s)	Add Attachment Delete Attachment View Attachment		

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EDUCATION AND TRAINING

B.S., Biology, Rensselear Polytechnic Institute
 Ph.D., Microbiology University of Massachusetts Amherst
 Post-Doctoral Research Associate with Dr. Derek R. Lovley, Department of Microbiology, University of Massachusetts Amherst
 Research Assistant Professor, Department of Microbiology, University of Massachusetts Amherst

2012-present Research Associate Professor, Department of Microbiology, University of Massachusetts Amherst

RELATED RESEARCH ARTICLES

- Nevin, K.P., T.L. Woodard, A.E. Franks, Z.M. Summers, and D.R. Lovley. 2010. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. mBio, doi:10.1128/mBio.00103-10.
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- Strycharz, S.M., S.M. Gannon, A.R. Boles, A.E. Franks, K.P. Nevin, D.R. Lovley. 2010, Reductive dechlorination of 2-chlorophenol by *Anaeromyxobacter dehalogenans* with an electrode serving as the electron donor. Environmental Microbiology Reports, 2: 289–294.
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- Ueki, T., Nevin, KP, Lovley DR. 2012. Engineering Clostridium ljungdahlii for acetone production. (manuscript in revision)
- Ueki T, Nevin KP, Leang C, Lovley DR. 2013. Deletion of a hydrogenase required for growth of Clostridium ljungdahlii on hydrogen provides evidence for direct electron transfer during microbial electrosynthesis. (manuscript submitted)
- Thomas, A. W., L. E. Garner, K. P. Nevin, T. L. Woodard, A. E. Franks, D. R. Lovley, J. J. Sumner, C. J. Sund, and G. C. Bazan. 2012. Membrane-intercalating conjugated oligoelectrolyte enables Shewanella oneidensis to use a graphite electrode as an electron donor. (manuscript submitted).
- Strycharz, S. M., R. H. Glaven, M. V. Coppi, S. M. Gannon, L. A. Perpetua, A. Liu, K. P. Nevin, and D. R. Lovley. 2011. Gene expression and deletion analysis of mechanisms for electron transfer from electrodes to *Geobacter sulfurreducens*. Bioelectrochemistry 80:142-150.
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- Zhang, T., S. M. Gannon, K. P. Nevin, A. E. Franks, and D. R. Lovley. 2010. Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. Environ. Microbiol. 12:1011-1020.

RELATED PATENTS:

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- Geobacteraceae Strains and Methods (patent application on genetic modification for higher current production)
- Microbial fuel cells (joint patent with Toyota)

Current Support

Kelly Nevin, University of Massachusetts

Electrofuels via Direct Electron Transfer from Electrodes to Microbes II U.S Department of Energy: Advanced Research Projects Agency - Energy

Jan 2011 – Jun 2013 4 months effort per year as Co-PI \$3,026,719

Mechanisms for Electron Transfer through Electrochemically Active Biofilms

Office of Naval Research

Jan 2010 - Dec 2012 4 academic months effort per year as Co-PI \$621,508

Real Time Monitoring of Rates of Subsurface Microbial Activity Associated with Natural Attenuation and Electron Donor Availability for Engineered Bioremediation with Current-Producing Microorganisms

U.S Department of Energy

Sept 2011 – Aug 2014 3 academic months effort per year as Co-PI \$1,001,671

Pending Support:

Rewiring a Microbial Chassis to Optimize Electrosynthesis

Office of Naval Research

4 years 4 academic months effort per year as Co-PI \$1,598,052

This proposal:

Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological Studies

Office of Naval Research

3 years 4 academic months effort per year as Co-PI \$605,513

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EDUCATION:

University of Connecticut	B.A.	1971-1975	Biological Sciences
Clark University	M.A.	1976-1978	Biological Sciences
Michigan State University	Ph.D.	1978-1982	Microbiology
Virginia Polytechnic Institute	Postdoctora	al 1982-1984	Microbiology

PROFESSIONAL APPOINTMENTS:

1999-Present: Distinguished University Professor, University of Massachusetts 2004-Present: Associate Dean, College of Natural Resources and the Environment

1997-2004: Department Head, Department of Microbiology

1995-1999: Professor, Department of Microbiology, University of Massachusetts

1984-1995: Research Hydrologist (GS-15), Water Resources Division, U.S. Geol. Survey

SELECT AWARDS:

2003-Present:	Most Highly Cited, Institute for Scientific Information (ISI H factor: 100)
2009:	Top 50 Invention for 2009, Time Magazine
2007:	Life Achievement Award, AHES Foundation
2007	'Top Cited Author', Environmental Microbiology
2006	Top contributors to biotechnology in the last decade, <i>Nature Biotechnology</i>
2004	Proctor and Gamble Award in Applied and Environmental Microbiology
2003	Time Magazine featured Environmental Innovator
1997	Fellow, American Academy of Microbiology
1992	Grand Winner, <i>Popular Science</i> , Best of What's New in Environmental
	Technology

RELEVANT PATENTS:

Microbial Production of Multi-Carbon Chemicals and Fuels from Water and Carbon Dioxide using Electric Current

Geobacteraceae Strains and Methods (patent application on genetic modification for higher current production)

Systems and Methods for Microbial Reductive Dechlorination of Environmental Contaminants (patent on use of cathode to promote reductive dechlorination)

Microbial fuel cells (joint patent with Toyota)

Microbial Nanowires Related Systems and Methods of Fabrication

SELECTED RELEVANT PUBLICATIONS (Microbe-Electrode Interactions 2010-2012)

Lovley D.R. and K.P. Nevin. 2013. Electrobiocommodities: Powering microbial production of fuels and commodity chemicals from carbon dioxide with electricity. Curr. Opin. Botechnol. (in press).

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Current Support

Derek R. Lovley, University of Massachusetts

Electrofuels via Direct Electron Transfer from Electrodes to Microbes II
U.S Department of Energy: Advanced Research Projects Agency - Energy
Jan 2011 – Jun 2013
2.5 months effort per year

\$3,026,719

Mechanisms for Electron Transfer through Electrochemically Active Biofilms

Office of Naval Research

Jan 2010 – Dec 2012 0.75 academic months effort per year

\$621,508

Coupled *In Silico* Microbial and Geochemical Reactive Transport Models: Extension to Multi-Organism Communities, Upscaling and Experimental Validation

U.S Department of Energy

May 2010 – May 2013

0.5 academic months effort per year

\$629,381

Mechanisms for Electron Transfer Through Pili to Fe(III) Oxide in Geobacter

U.S Department of Energy

June 2010 – May 2013

0.5 academic months effort per year

\$814,534

Systems Level Analysis of the Function and Adaptive Responses of Methanogenic Consortia U.S Department of Energy

Jul 2010 – Jun 2013

1 academic months effort per year

\$1,220,828

Real Time Monitoring of Rates of Subsurface Microbial Activity Associated with Natural Attenuation and Electron Donor Availability for Engineered Bioremediation with Current-Producing Microorganisms

U.S Department of Energy

Sept 2011 – Aug 2014

0.5 academic months effort per year

\$1,001,671

Mechanisms Underlying the Metallic-Like Conductivity of Microbial Nanowires

Office of Naval Research

Jan 2012 - Dec 2014

0.75 academic months effort per year

\$638,349

Pending Support:

Rewiring a Microbial Chassis to Optimize Electrosynthesis

Office of Naval Research

4 years 0.75 academic months effort per year

\$1,598,052

This proposal:

Factors Limiting Power Output of Benthic Microbial Fuel Cells: Mechanistic and Ecological Studies

Office of Naval Research

3 years 0.6 summer months effort per year \$605,513